Algal Symbiosis in *Bunodeopsis:* Sea Anemones with "Auxiliary" Structures

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Abstract. This study describes the photobiology of two tropical species of the symbiotic sea anemone genus *Bunodeopsis* from Discovery Bay, Jamaica. *B. antilliensis* was found in shallow water (0.3 m) and experienced higher irradiance levels than *B. globulifera* from deeper water (3 m). Both species contained symbiotic dinoflagellates of the genus *Symbiodinium* within the endodermal cells. The external morphology and expansion-contraction behavior of the two anemone species were closely linked to symbiont distribution. *B. antilliensis* had large vesicles (2.6 mm³), with 88.5% of the symbiont population in the lower column and basal disk and 11.5% in the tentacles and upper column, and was contracted under normal daylight illumination. In contrast, *B. globulifera* had small vesicles (0.2 mm³), with 55.5% of the symbionts in the lower column and basal disk and 44.5% in the tentacles and upper column, and was expanded under illumination.

The photosynthetic physiology of the symbionts indicated that those from *B. globulifera* were adapted to lower host habitat irradiances than were those from *B. antilliensis*. The symbionts from *B. globulifera* had a significantly higher chlorophyll *a* content (7.34 ± 0.77 pg · cell⁻¹) and photosynthetic efficiency (0.24 μgO$_2$ · 10⁶ cells · h⁻¹/ μmol photons · m⁻² · s⁻¹) and lower saturation irradiance (277 ± 18 μmol photons · m⁻² · s⁻¹) than those from *B. antilliensis*, 4.51 ± 0.29 pg · cell⁻¹, 0.17 μgO$_2$ · 10⁶ cells · h⁻¹/μmol photons · m⁻² · s⁻¹ and 436 ± 78 μmol photons · m⁻² · s⁻¹, respectively. The calculated rate of carbon translocation in both species of *Bunodeopsis* (97%) was high and reflected the low algal protein biomass ratios (2%) and population growth rates (<0.1 · day⁻¹). The CZAR values in *B. antilliensis* (109%) and *B. globulifera* (92%) suggest that both species are potentially autotrophic with respect to carbon available for animal respiration.

Introduction

Most tropical marine Cnidaria, including sea anemones and corals, contain symbiotic dinoflagellate algae of the genus *Symbiodinium* (Trench and Blank, 1987), also known as "zooxanthellae." These algal symbionts are generally located within endodermal cells and are nutritionally important to the associations because they are photosynthetic and much of the fixed carbon is translocated to the cnidarian host (Muscatine and Cernichiari, 1969; Muscatine *et al.*, 1983). The photosynthetic capacity of the symbionts is flexible and varies with host irradiance regime; therefore, animal and algal responses that enhance symbiont exposure to light and photosynthesis will be beneficial to the whole association.

Tropical marine Cnidaria display a diverse array of responses related to the possession of symbionts. Morphological adaptations to symbiosis are illustrated by the "auxiliary" structures of symbiotic sea anemones, which contain high densities of symbiotic algae relative to other body regions (Sebens and deReimer, 1977). Expansion of these structures under illumination maximizes exposure of the symbiont populations to light, thereby enhancing their photosynthesis. Examples of auxiliary structures include the "pseudotentacles" of *Lebrunia coralligens, L. danae* (Gladfelter, 1975), and *Phyllodiscus semoni* (Shick *et al.*, 1991); the diskal tentacles of *Discosoma sanctithomae* (Elliott and Cook, 1989); and the collar of *Phyllactis flosculifera* (Steele and Goreau, 1977).

The algal symbionts experience variations in irradiance regime due to host habitat, morphology, and behavior. Algal symbionts display compensatory responses to maximize photosynthesis; these include variations in photo-

synthetic pigment content and physiology, which may be assessed by parameters of photosynthesis-irradiance (P-I) curves (Chalker, 1981). The nutritional contribution made by the symbiont population to the host may be estimated by the index CZAR, or "the contribution of translocated zooxanthellal carbon to the daily animal respiratory carbon requirements" (Muscatine *et al.*, 1981). A high proportion of photosynthetically fixed carbon is translocated to the host, as shown in the symbiotic sea anemones *Aiptasia pallida* (Clayton and Lasker, 1984) and *Anemonia sulcata* (Stambler and Dubinsky, 1987), and this represents a large contribution to the host's respiratory needs (reviewed in Shick, 1991). The nutritional contribution made by algal symbionts to these sea anemones also varies with the extent of host heterotrophy, zooplankton feeding, or uptake of dissolved nutrients (Zamer, 1986). Investigations of algal and animal responses in symbiotic sea anemones include those for the tropical species *Aiptasia pulchella* (Muller-Parker, 1984, 1985, and 1987; Steen, 1986), *Aiptasia pallida* (Cook *et al.*, 1988; Lesser and Shick, 1989), and *Phyllodiscus semoni* (Shick *et al.*, 1991), and for the temperate species *Anthopleura elegantissima* (Fitt *et al.*, 1982; Shick and Dykens, 1984; Zamer and Shick, 1987) and *Anemonia sulcata* (= *viridis*) (Taylor, 1969; Tytler and Davies, 1984).

This study presents the first detailed description of animal and algal responses that enhance the photosynthesis and the nutritional contribution of the symbionts in the sea anemone *Bunodeopsis* (Boloceroididae; Carlgren, 1924). This genus is of particular interest because these sea anemones have auxiliary structures (Sebens and deReimer, 1977) that contain high densities of symbiotic algae (Hyman, 1940). *B. antilliensis* (Duerden, 1897) and *B. globulifera* (Verrill, 1900) occupy different habitats in terms of depth and irradiance regime. These two species also have distinctly different morphologies and light-related patterns of expansion and contraction behavior. The relationship between these environmental factors and symbiont distribution, photosynthetic pigment content, and physiology of the two species of *Bunodeopsis* is examined in relation to CZAR. Lastly, the extent of zooplankton feeding by these two species of *Bunodeopsis* is investigated in a 24-h *in situ* study.

Materials and Methods

Collection and maintenance

Specimens of *Bunodeopsis* were observed *in situ* and collected from *Thalassia testudinum* beds in Discovery Bay, Jamaica (Lat. 77°25' W; Long. 18°30' N) during studies conducted between January 1989 and 1991. Whole *Thalassia testudinum* blades, with the anemones adhering, were placed into self-sealing plastic bags with seawater, and then transferred to glass aquaria (61 × 31 × 30 cm).

The aquaria were covered with fine mesh (2-mm mesh size) to contain the sea anemones and placed on tables of flowing seawater. Every second night, anemones were fed to repletion with freshly hatched *Artemia salina* nauplii, applied by pipette directly to the tentacles. Anemones maintained in otherwise constant darkness were exposed to light for less than 5 min during each feeding. Aquaria were cleaned several times each week to prevent fouling.

Morphology and histology

Morphological measurements were made on contracted anemones attached by their basal disks to petri dishes at 50 × under a dissecting microscope with a calibrated ocular micrometer. Basal disk diameter, width, and total number of vesicles were measured for individual anemones. For histological preparations, anemones were narcotized in 7.5% (w/v) $MgCl_2 \cdot 6H_2O$ solution and fixed in Bouin's solution in seawater. Preparation of anemones for transmission electron microscopy was conducted as described by Parke and Manton (1967). Silver-to-gold sections were stained with a saturated solution of uranyl acetate in 50% ethanol and Reynold's lead citrate, and viewed in a Phillips EM 400 transmission electron microscope.

Biomass parameters

Animal and algal biomass parameters were determined for whole anemones and separate body regions for specimens collected in November 1989. Anemones were relaxed in darkness for 10–15 min. at 4°C and narcotized in 7.5% $MgCl_2 \cdot 6H_2O$ (w/v) solution prior to dissection into four body regions (tentacles, capitulum, scapus, and basal disk) at 50 × under a dissecting microscope. Whole anemones or separate body regions were homogenized in Millipore-filtered (0.22-μm pore size) seawater, and the animal and algal fractions were separated by centrifugation as described by Muller-Parker (1984). The algal pellet was resuspended in filtered seawater, and cell numbers were determined in a hemacytometer at 400 ×. Protein content of the animal fraction was assayed by the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

Algal cell size was determined from the diameter of dividing cells, measured parallel to the plane of division at 500 × with a calibrated ocular micrometer, for 10 cells per anemone from eight anemones of each species. Cell volumes were calculated from cell diameters, and the mean cell carbon content was estimated from the equations of Strathmann (1967) determined for phytoplankton (including dinoflagellates and excluding diatoms). From the mean algal density and the weight of carbon per cell, the standing stock of algal carbon was calculated. Total algal protein was estimated as (6.25 × C)/C:N, and the

ratio of algal to "total" (animal plus algal) protein was calculated using a C:N ratio of 9.4:1 (Cook *et al.,* 1988).

To determine whether the algal symbionts had a phased cell division cycle, the mitotic index (number of cells dividing per 100) was recorded for algae isolated from anemones at intervals during a 24-h period. Anemones were freshly collected from study sites every 2 h between 0800 and 1800 h. For nighttime studies, anemones were sampled every 3 h between 2000 and 0600 h from anemones that had been collected from study sites at 1800 and placed into aquaria of flowing seawater (Wilkerson *et al.,* 1983; Fitt and Trench, 1983). The algae were immediately separated from the animal fraction, resuspended in 5 ml of 5% formalin in seawater (v/v), and refrigerated at 4°C until cell counts were made. Three replicate counts were made for three anemones of each species during each sampling time. Chlorophylls *a* and c_2 of the algal symbionts were assayed following the method outlined by Muller-Parker (1984).

Expansion and contraction behavior

Anemones were observed *in situ* at collection sites between January and March 1990, and in aquaria under laboratory conditions. Anemones were considered to be in an "expanded" posture if the oral disk was expanded and the tentacles extended. In "contracted" anemones, the oral disk was contracted, with the tentacles retracted into the gastrovascular cavity, the basal disk extended, and the vesicles inflated. Anemones in intermediate postures were rarely observed. At the study sites, percentage transmission of surface irradiance to depth was recorded as photosynthetically active radiation (PAR: 400–700 nm) with a cosine-corrected Li-Cor quantum sensor (Model 192) and Li-Cor photometer (Model LI 185A). Underwater measurements were made with the meter contained in a clear acrylic housing sealed with an o-ring. *In situ,* the postures of a minimum of 50 anemones and irradiance were noted three times daily at about 0700, 1200, and 1900 h, twice weekly for a period of 8 weeks.

Under laboratory conditions, irradiance was provided by lighting from nearby windows with artificial illumination from overhead cool-white fluorescent lamps (12 h d^{-1}, approx. 100 μmol photons$\cdot m^{-2} \cdot s^{-1}$). Anemones having low symbiont densities were obtained by the method of "cold-stripping" (Steen and Muscatine, 1987). Anemones in seawater were placed into a refrigerator at 4°C for 30 min until the seawater temperature was reduced to about 16°C (when cooled below 16°C, all the anemones died), then transferred to aquaria of flowing seawater (28°C) and maintained in darkness for 2 weeks. Expansion and contraction behavior was observed for two groups of untreated and cold-stripped anemones, which were placed under laboratory irradiance and in constant darkness. The postures of the anemones were noted at 0700, 1200, and 1900 h daily for 2 weeks. At the termination of these studies, biomass parameters were determined for individual anemones from each treatment.

Zooplankton feeding

The extent of zooplankton feeding was assessed by examining coelenteron contents from 10 anemones of each species collected from study sites every 3 h for 24 h. This time interval was chosen because zooplankton prey can be completely digested within 4 to 6 h of capture (Sebens and Koehl, 1984). Nocturnal collections were essential because peak zooplankton activity occurs within 2 h of sunset in Discovery Bay (Ohlhorst, 1982). The anemones were allowed to expand in darkness, then removed from the *Thalassia testudinum* blades and transferred to plastic bags filled with 10% formalin in seawater (v/v). The basal disk diameters were measured; the coelenteron contents removed; and the number, maximum length, and type of each recognizable prey item (Newell and Newell, 1966) recorded at 50 × with a dissecting microscope and calibrated ocular micrometer.

Photosynthesis-irradiance relationships

Oxygen flux was recorded for anemones, within 2 h of collection, in a cylindrical clear acrylic chamber (75-ml volume) fitted with a Clark Beckman 1000 microcathode connected to a Linear chart recorder. Constant temperature (28°C) of the chamber seawater was maintained by a water jacket receiving water from a recirculating water bath. Even mixing of the water column, without disturbance to the anemone, was ensured by a pin-mounted magnetic stirrer. The chamber was positioned beneath a variable light source, consisting of a 250-W tungsten lamp projected through Corning 3405 and 3409 (50 mm²) filters, with an 80B Kodak filter to convert incandescent light to a more "natural" spectrum experienced at depths of less than 5 m. The light source was calibrated with the Li-Cor quantum sensor (Model 192) and Li-Cor photometer (Model LI 185A) used in the behavioral studies. Two fans directed at the bulb and a heat filter prevented the light source from increasing the chamber temperature.

The electrode was calibrated between incubations with air-saturated seawater and oxygen-free seawater (with sodium sulphite). The oxygen concentration of seawater was reduced to between 50 and 60% air saturation by bubbling with gaseous nitrogen. Low-oxygen seawater was added to the chamber seawater during incubations to maintain a concentration of 75 to 100% air saturation, and chamber seawater was replaced during each reading. As a control for biological activity of the incubation medium and oxygen consumption by the cathode, oxygen flux was re-

corded for seawater alone in darkness for 30 min. No oxygen uptake was recorded for three such replicates.

Specimens of *B. globulifera* anemones were placed directly into the chamber, where they attached rapidly to the base. Because *B. antilliensis* required several hours for attachment, specimens were allowed to settle on pieces of plastic sheet, which were subsequently transferred to the base of the chamber. After a 5-min equilibration period, oxygen flux was recorded for 20 min at consecutive ascending irradiances 10, 30, 100, 200, 300, 400, and 500 μmol photons $\cdot$ m$^{-2} \cdot$ s^{-1}. In darkness, both species of anemone rapidly expanded. However, because *B. globulifera* anemones contracted much more rapidly under illumination than did *B. antilliensis* anemones, dark respiration rates were recorded at the beginning of incubations for *B. globulifera* and at the end of incubations, following a 20-min equilibration period, for *B. antilliensis*. Anemone biomass parameters were assessed at the termination of each incubation.

Oxygen flux measurements were standardized to sea anemone protein and to algal cell number for 10 specimens of *B. antilliensis* and 12 of *B. globulifera*. Hyperbolic tangent function curves (Chalker, 1981) were fitted to the net photosynthesis *versus* irradiance data with a least-squares regression analysis program. Mean P-I characteristics, photosynthetic capacity (P_{max}), photosynthetic efficiency (α), and I_k (the irradiance at which the initial slope of the curve (α) intercepts the horizontal asymptote) were derived for each species from the individual P-I curves. Saturation irradiance ($I_{0.95}$) was estimated from the irradiance at which photosynthesis was 95% of the maximum, where $I_{0.95} = 1.83 \times I_k$ (Chalker *et al.*, 1983).

CZAR index

The potential contribution of algal photosynthetically fixed carbon to animal respiratory requirements was estimated as CZAR = $(P_Z^N \times T)/R_A$ (Muscatine *et al.*, 1981), where P_Z^N is the total daily net carbon fixed by the algae, T is the fraction of P_Z^N translocated to the host, and R_A is the daily respiration of the animal in carbon equivalents. Respiration rates of the animal and algal components were assumed to be proportional to their measured and derived protein biomasses respectively (Muscatine *et al.*, 1983). Oxygen consumed in respiration was converted to carbon equivalents by using the respiratory quotient (RQ) multiplied by the molecular weight conversion factor of 0.375. The algal respiration rate was estimated as $r_Z = 1 - \beta(r_{Anemone}) \times 0.375$ RQ$_{Anemone}$ and the animal respiration rate as $R_A = \beta(r_{Anemone})$, where $r_{Anemone}$ is the measured anemone rate of dark respiration and β is the protein biomass ratio. The anemone respiratory quotient calculated as RQ$_{Anemone}$ = $[((1 - \beta)/RQ_Z) + \beta/RQ_A)]^{-1}$ was 0.8 for both *B. antilliensis* and *B. globulifera*. The algal

and animal respiratory quotients (RQ$_Z$ and RQ$_A$) were assumed to be 0.8 and 1.0, respectively (Muscatine *et al.*, 1981).

Total daily net carbon fixation by the algae (P_Z^N) was estimated as the gross photosynthetic capacity of the anemone (p_{max}^G) minus the derived algal respiration rate (r_Z), multiplied by the daily number of hours at saturation irradiance ($I_{0.95}$). For comparative purposes, the contribution made by symbiont photosynthesis at irradiances below saturation was assumed to be negligible (see Fig. 9). *In situ* irradiances were predicted from mean transmission of surface PAR to the study site depths, together with surface PAR recordings made at 30-min intervals on 22 February 1990 (with a maximum irradiance of 2100 μmol photons $\cdot$ m$^{-2} \cdot$ s^{-1}). The gross photosynthetic capacity of the anemone (p_{max}^G) was equal to the measured net photosynthetic capacity (p_{max}^N) plus the dark respiration rate of the anemone ($r_{Anemone}$), converted to carbon equivalents, assuming a photosynthetic quotient of 1.1 (Muscatine *et al.*, 1981).

The proportion of fixed carbon translocated from the symbionts to the host (T) was estimated by the growth rate method of Muscatine *et al.* (1983), using the equation T = $\mu_c - \mu/\mu_c \times 100\%$, where μ and μ_c are the algal and carbon specific growth rates, respectively. The algal population growth rate (μ) was derived from the mitotic index (Biomass parameters) using the equation of Wilkerson *et al.* (1983). The carbon-specific growth rate was calculated as $\mu_c = 1/C' \times \delta C/\delta t$, where C' is the standing stock of algal carbon (Sea anemone biomass parameters), and $\delta C/\delta t$ is the total net daily carbon fixed by the algae (P_Z^N). Translocation was not measured directly with ^{14}C, because the use of this radioactive isotope is known to underestimate carbon translocation *in vivo* (Muscatine *et al.*, 1983).

Statistical analysis

Data were analysed by the following parametric tests: Student's *t* test; two-factor analysis of variance (ANOVA); Scheffé's and Tukey-Kramer multiple range tests; and correlation analysis (Sokal and Rohlf, 1981). Nonparametric tests used were Wilcoxon's signed ranks; Mann-Whitney *U*; and χ^2 tests (Siegel and Castellan, 1988). Angular transformations (Sokal and Rohlf, 1981) were used on all percentage data prior to statistical analysis.

Results

Habitat

Bunodeopsis antilliensis (Figs. 1a, 2) was found on *Thalassia testudinum* blades in water shallower than 0.3 m in the West back reef and *B. globulifera* (Fig. 1b) at 3 m depth in the East back reef area of Discovery Bay, Jamaica.
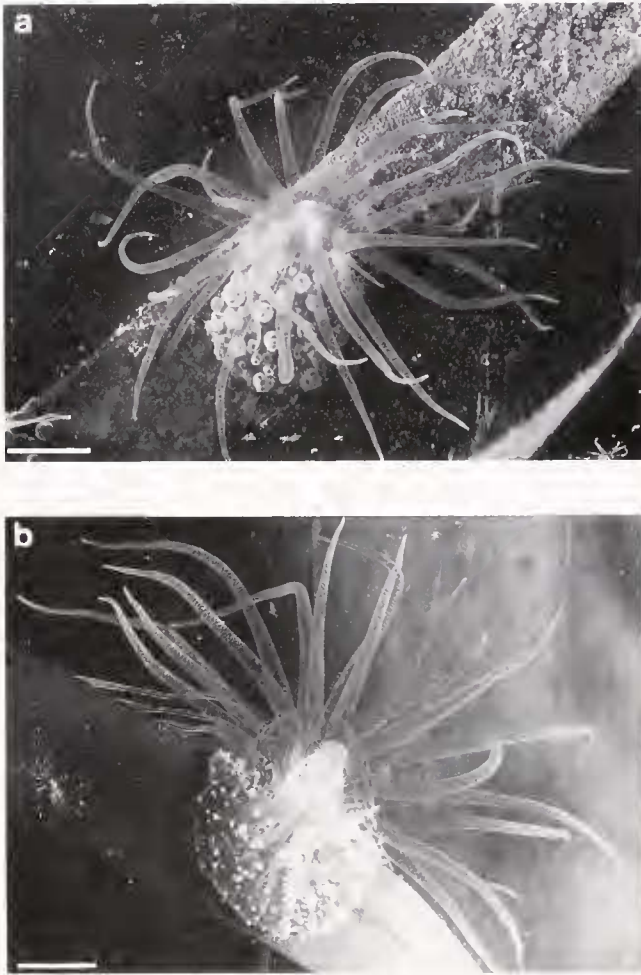
Figure 1. (a) *Bunodeopsis antilliensis* and (b) *B. globulifera* in expanded postures. Scale bar = 1 cm.

B. antilliensis anemones experienced higher ambient irradiances than *B. globulifera*, receiving 53 ± 2 and 26 ± 2% of surface irradiance (means ± SE; $N = 87$), respectively ($t_{(85)} = 11.23$; $0.01 < p > 0.05$).

Morphology and histology

Of the anemones collected, the larger specimens were *B. antilliensis* rather than *B. globulifera*; the basal disk diameters of the two species ranged from 5 to 34 mm and 4.4 to 15.2 mm, respectively. However, vesicle size (diameter) was not related to body size for either *B. antilliensis* ($r^2_{(10)} = 0.36$; $p > 0.05$) or *B. globulifera* ($r^2_{(10)} = 0.17$; $p > 0.05$). The vesicles of *B. antilliensis* (Fig. 2) were significantly larger than those of *B. globulifera*, with diameters (means ± SE) of 1.7 ± 0.2 and 0.7 ± 0.1 mm, respectively ($t_{(14)} = 4.17$; $0.01 < p < 0.05$). Assuming the vesicles to be spherical, this would represent mean volumes per vesicle of 2.6 mm³ for *B. antilliensis* and 0.2

mm³ for *B. globulifera*. Because the number of vesicles per anemone was directly related to body size for *B. antilliensis* ($r^2_{(10)} = 0.96$; $0.01 < p < 0.05$) but not for *B. globulifera* ($r^2_{(11)} = 0.05$; $p > 0.05$), the mean number of vesicles per anemone was not compared between species.

Light microscopy of paraffin sections demonstrated that the algal symbionts of *Bunodeopsis* were restricted to the endoderm, and were absent from the mesoglea and ectoderm. Transmission electron microscopy (Fig. 3) revealed that each algal cell was contained within a peri-symbiont space, bounded by multiple membranes of host origin, typical of Cnidaria-dinoflagellate symbioses (Trench, 1971). The most obvious dinoflagellate ultrastructural features were the permanently condensed chromosomes in the nucleus (Dodge, 1973). Other distinguishing dinoflagellate characteristics included a single or multistalked pyrenoid surrounded by a starch sheath and groups of three thylakoids per lamella within the chloroplast. The algal symbionts of *B. antilliensis* and *B. globulifera* had a single or multilobed chloroplast and an accumulation body, which are characteristic of *Symbiodinium* (Blank, 1987). Invasive thylakoid membranes within the pyrenoid and a segmented starch sheath (Dodge, 1973) were not observed in the symbionts of *B. antilliensis* or *B. globulifera*. The symbionts of *B. antilliensis* and *B. globulifera* were not identified to species level.

Biomass parameters

The measured and derived algal and animal biomass parameters of *B. antilliensis* and *B. globulifera* are shown in Table I. The two species of *Bunodeopsis* had similar densities of algal symbionts ($t_{(10)} = -0.7$; $p > 0.05$), which were of a similar size ($t_{(14)} = -1.29$; $p > 0.05$). The estimated standing stock of algal cell carbon and biomass ratios did not vary between species. However, the distri-

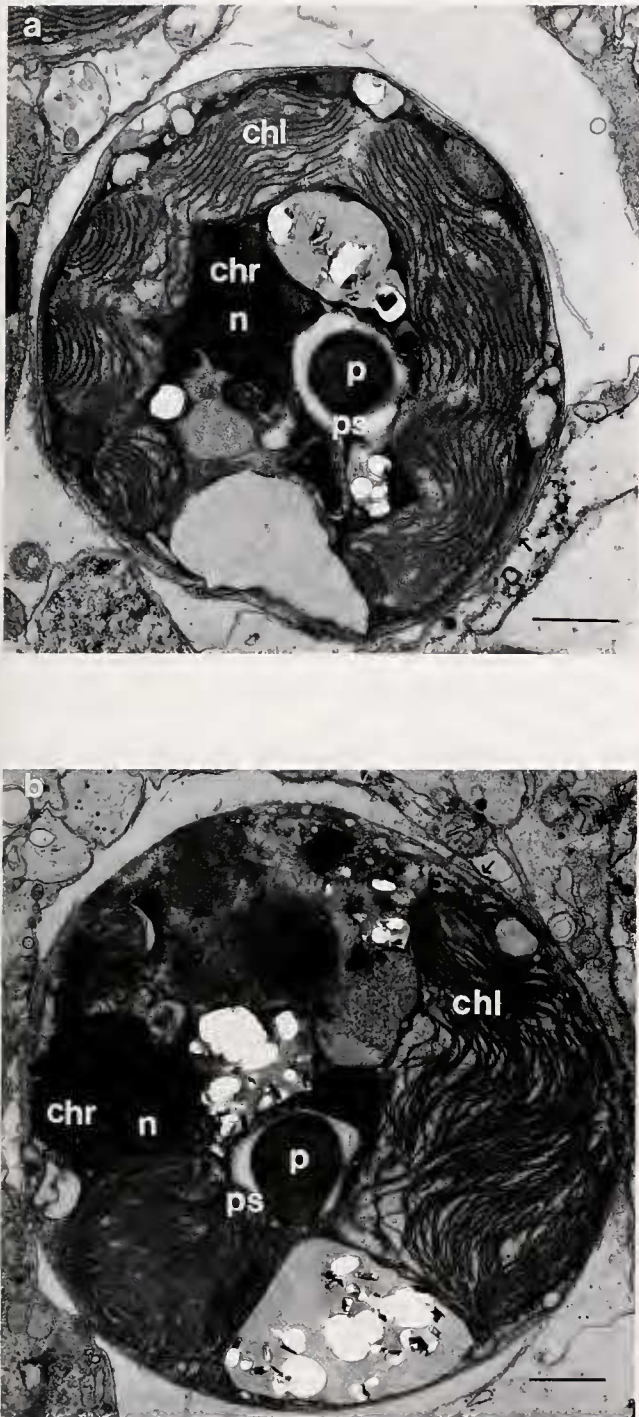**Figure 2.** *Bunodeopsis antilliensis* in contracted posture, showing inflated vesicles (V). Scale bar = 1 cm.

Figure 3. *Symbiodinium* in the vesicle endoderm of (a) *Bunodeopsis antilliensis* and (b) *B. globulifera* showing multiple membranes (↑); nucleus (n); chromosomes (chr); chloroplast (chl); pyrenoid (p); and pyrenoid stalk (ps). Scale bar = 1 μm.

bution of symbionts within the anemones was markedly different between the two species of *Bunodeopsis* (Fig. 4) (Two-factor ANOVA: $F_{(species)(1,40)} = 1.37$, $p > 0.05$;

$F_{(body\ region)(3,40)} = 368.42$; $0.01 < p < 0.05$; $F_{(interaction)(3,40)} = 103.10$; $0.01 < p < 0.05$). *B. globulifera* anemones had a higher proportion of symbionts in the tentacles (44%) than *B. antilliensis* (10%), which had most symbionts in the scapus and basal disk (85%) (Tukey-Kramer multiple comparisons test; $0.01 < p < 0.05$).

The diel cycles of symbiont cell division were phased in both *B. antilliensis* and *B. globulifera* (Fig. 5), with peak mitotic indices in the early morning. Symbionts from *B. globulifera* had a higher chlorophyll *a* content, 7.34 ± 0.77, than those from *B. antilliensis*, 4.51 ± 0.29 pg · cell^{-1} ($t_{(8)} = 3.45$; $0.01 < p < 0.05$). Chlorophyll c_2 content did not differ between the symbionts of *B. antilliensis* and *B. globulifera*, with 3.3 and 3.9 pg · cell^{-1}, respectively ($t_{(8)} = 1.19$; $p > 0.05$). The ratio of chlorophylls $a:c_2$ was higher for symbionts from *B. globulifera*, 1.88 ± 0.06, than from *B. antilliensis*, 1.40 ± 0.05 ($t_{(8)} = 7.46$; $0.01 < p < 0.05$).

Expansion and contraction behavior

In situ, almost all anemones of both species of *Bunodeopsis* were expanded at night, although marked differences in expansion and contraction behavior were observed during the day (Fig. 6). Under illumination, *B. antilliensis* anemones were contracted, whereas *B. globulifera* remained expanded, except at very high irradiances experienced at midday. The proportion of *B. globulifera* anemones in an expanded posture was found to be inversely related to irradiance (Fig. 7; $r^2_{(29)} = -0.69$; $0.01 < p < 0.05$). Under laboratory conditions of irradiance, untreated and cold-stripped anemones displayed patterns of expansion and contraction behavior similar to those observed *in situ* (Fig. 8). However, significantly more cold-stripped *B. globulifera* anemones than untreated anemones were contracted during the day (Scheffé's; $0.01 < p < 0.05$). In constant darkness, almost all anemones of both species were expanded, and the proportion of contracted *B. globulifera* was significantly higher for cold-stripped anemones than for the untreated group (Scheffé's; $0.01 < p < 0.05$). Cold-stripped anemones contained significantly fewer symbionts than untreated anemones, with densities (mean ± SE) of 0.03 ± 0.01 × 10^6 cells · mg protein^{-1} for *B. antilliensis* ($t_{(10)} = 13.19$; $0.01 < p < 0.05$), and 0.26 ± 0.05 × 10^6 cells · mg protein^{-1} for *B. globulifera* ($t_{(8)} = 6.51$; $0.01 < p < 0.05$). "Juvenile" anemones (with a basal disk diameter of less than 5 mm), which were formed by pedal laceration from "adults," were continually expanded, both under illumination and in darkness.

Zooplankton feeding

Prey items were found at various stages of digestion within the gastrovascular cavity, mesentery, and oral disk regions of *B. antilliensis* and *B. globulifera*. The compo-

Table I

Sea anemone biomass parameters of Bunodeopsis antillinesis *and* B. globulifera

Parameter	Method*	B. globulifera	B. antilliensis
Algal cell diameter (μm) (mean $\pm$ SE; $N = 8$)	M	7.95 $\pm$ 0.70	7.60 $\pm$ 0.34
Algal cell density ($\times 10^6 \cdot$ mg protein^{-1}) (mean $\pm$ SE; $N = 6$)	M	0.61 $\pm$ 0.04	0.65 $\pm$ 0.02
Algal carbon (pg $\cdot$ cell^{-1})	D	43.2	38.4
Standing stock of algal carbon (μg $\cdot$ mg protein^{-1})	D	26.4	25.0
Algal protein (pg $\cdot$ cell^{-1})	D	28.7	25.5
Protein biomass ratio (animal:total)	D	0.98	0.98
Peak mitotic index (%) (mean $\pm$ SE; $N = 3$)	M	5.0 $\pm$ 2.6	8.0 $\pm$ 1.9
Algal population growth rate (μ) (day^{-1})	D	0.05	0.08
Carbon specific growth rate (μ_c) (day^{-1})	D	1.90	2.56

* M = measured; D = derived.

sition of prey ingested by the two species was similar ($\chi^2_{(7)} = 9.14$; $p > 0.05$, for nighttime feeding), with Crustacea the most common prey type (Table II). Crustacea represented 90% and 84% of the total numbers of prey recorded for *B. antilliensis* and *B. globulifera*, respectively. Other prey types included polychaetes, mollusks, and urochordates. Although *B. antilliensis* anemones sampled were larger (mean basal disk diameter $\pm$ SE), 7.8 $\pm$ 0.29 mm, than *B. globulifera*, 6.2 $\pm$ 0.29 mm ($Z = 4.49$; $0.01 < p < 0.05$), no significant correlation was found between anemone size and number of prey caught for *B. antilliensis* ($r^2_{(70)} = 0.09$; $p > 0.05$) or *B. globulifera* ($r^2_{(70)} = 0.02$; $p > 0.05$). Similar total numbers of prey items

were found in the 80 anemones of each species sampled during the 24-h period; 83 in *B. globulifera* and 88 in *B. antilliensis* (Wilcoxon's signed rank test for paired samples; $T_s = 16$; $p > 0.05$), which were also of a comparable size ($Z = 0.80$; $p > 0.05$). Both species ingested significantly more prey items at night than during the day (Mann-Whitney U test; $0.01 < p < 0.05$). At night, prey ingestion did not vary between species (Mann-Whitney U test $p > 0.05$); during the day, however, *B. globulifera* ingested significantly more prey than *B. antilliensis* (Mann-Whitney U test; $0.01 < p < 0.05$).

Photosynthesis-irradiance relationships and CZAR

P-I curves for *B. antilliensis* and *B. globulifera* are shown in Figure 9. Both species of *Bunodeopsis* had sim-
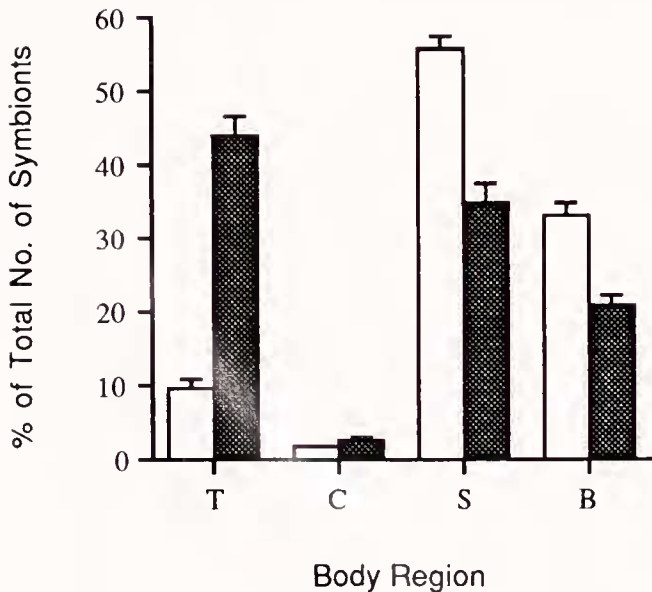
Figure 4. Distribution of symbionts within body regions; tentacles (T), capitulum (C), scapus (S) and basal disk (B) of *Bunodeopsis antilliensis* (open bars) ($N = 7$) and *B. globulifera* (solid bars) ($N = 5$). Values are means $\pm$ SE.
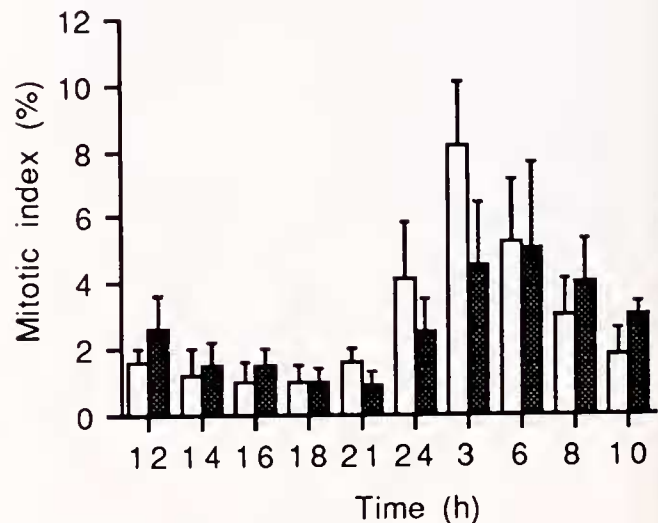
Figure 5. The diel cycle of symbiont cell division from *Bunodeopsis antilliensis* (open bars) and *B. globulifera* (solid bars). Values are means $\pm$ SE ($N = 3$).
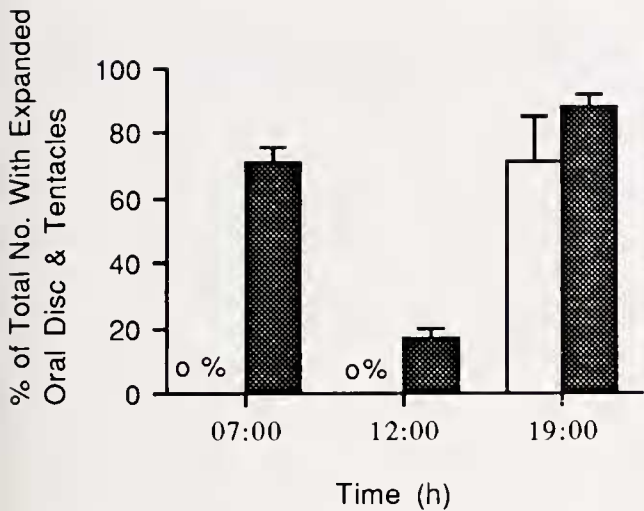
Figure 6. Oral disk and tentacle expansion and contraction in *Bunodeopsis antilliensis* (open bars) and *B. globulifera* (solid bars) *in situ*. Two-factor ANOVA: $F_{(species)(1,52)} = 42.83^*$; $F_{(time)(2,52)} = 51.43^*$; $F_{(interaction)(2,52)} = 10.46^*$ (*significant at $0.01 < p < 0.05$). 0% = No *B. antilliensis* anemones with expanded oral disk and tentacles.

ilar dark respiration rates ($r_{Anemone}$) and gross photosynthetic capacities (p_{max}^G) (Table III). The photosynthetic efficiencies (α; means ± SE) were 0.24 ± 0.02 for *B. globulifera* and 0.17 ± 0.03 $\mu gO_2 \cdot 10^6$ algae$^{-1} \cdot h^{-1}/\mu$mol photons $\cdot m^{-2} \cdot s^{-1}$ for *B. antilliensis*. However, the deeper living species *B. globulifera* attained photosynthetic capacity at a significantly lower saturation irradiance ($I_{0.95}$;
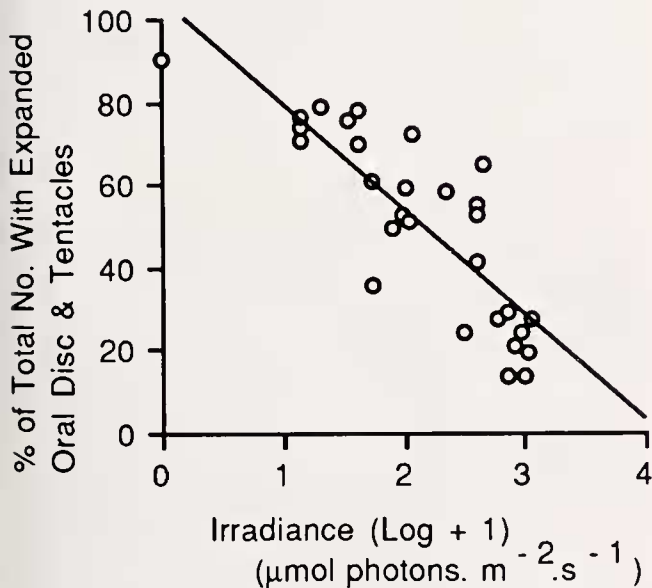

Figure 7. Variation of oral disk and tentacle expansion with irradiance in *Bunodeopsis globulifera*. Points were fitted by the method of least squares regression analysis.
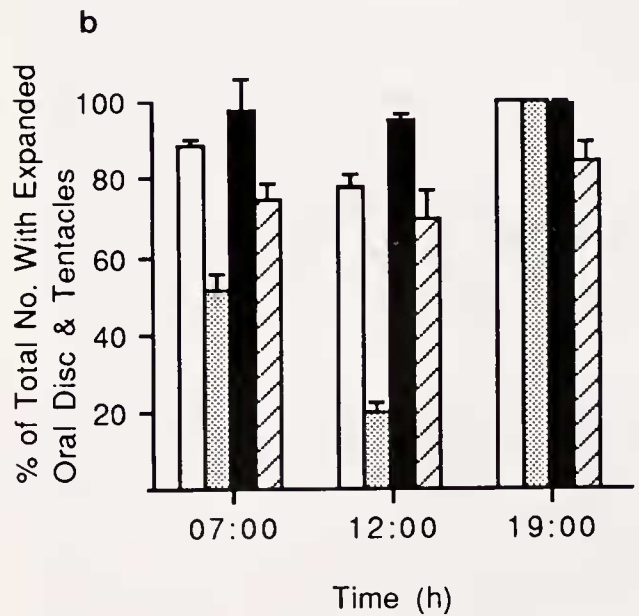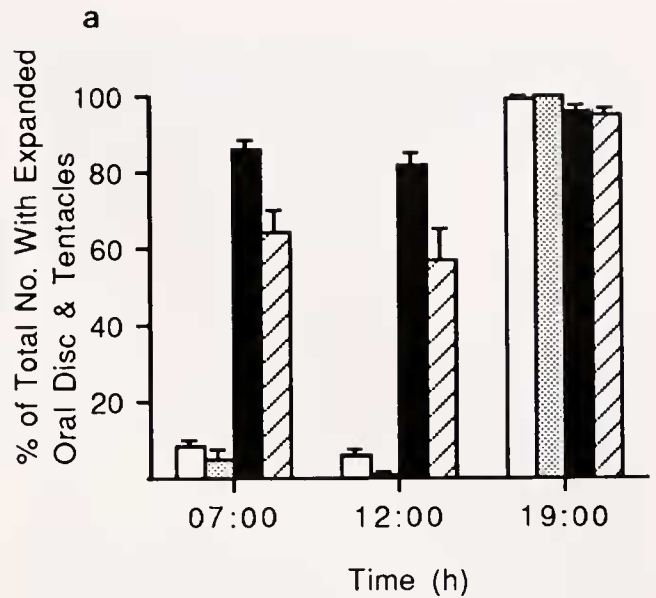

Figure 8. Oral disk and tentacle expansion and contraction in (a) *Bunodeopsis antilliensis* and (b) *B. globulifera* in aquaria under four treatment groups: untreated under ambient irradiance (open bars) and in constant darkness (solid bars); and cold-stripped under ambient irradiance (shaded bars) and in constant darkness (hatched bars). (a) Two-factor ANOVA; $F_{(treatment)(3,43)} = 139.24^*$; $F_{(time)(2,143)} = 425.25^*$; $F_{(interaction)(6,143)} = 50.31^*$. (b) Two-factor ANOVA; $F_{(treatment)(e,43)} = 89.41^*$; $F_{(time)(2,143)} = 117.67^*$; $F_{(interaction)(6,143)} = 27.38^*$ (*significant at $0.01 < p < 0.05$).

mean ± SE), 227 ± 18, than *B. antilliensis*, 436 ± 78 μmol photons $\cdot m^{-2} \cdot s^{-1}$, ($t_{(20)} = -2.85$; $0.01 < p < 0.05$). *B. globulifera* and *B. antilliensis* both experienced about 8.5 h of saturating irradiance as calculated from the diel cycle of PAR (Fig. 10), *in situ* measurements of transmission of surface irradiance to study site depth, and sat-

Prey Item	B. antilliensis		B. globulifera	
	Day	Night	Day	Night
Crustacea				
Ostracoda	0	3	0	3
Copepoda	0	24	7	16
Decapoda	0	13	7	10
Brachyura	0	22	1	5
Isopoda	0	2	2	3
Amphipoda	2	13	6	10
Non-Crustacea				
Polychaeta	0	1	1	2
Other	0	8	0	10

of symbionts from other tropical sea anemones (Steen and Muscatine, 1984; Smith, 1986; Cook *et al.*, 1988). The peak mitotic indices of symbionts from *B. antilliensis* (8%) and *B. globulifera* (5%) were similar to those from fed *Aiptasia pallida* (7 to 8%) (Cook *et al.*, 1988), but much higher than those from *Zoanthus sociatus, Palythoa variabilis* (both 0.6%; Steen and Muscatine, 1984), and *A. pulchella* (0.3 to 1.1%; Muller-Parker, 1984, 1985, 1987; Wilkerson *et al.*, 1983; Steen, 1986). Consequently the population doubling times of symbionts from *B. antilliensis, B. globulifera,* and *A. pallida* (Cook *et al.*, 1988) were much shorter, 8 to 12 days, than those from *Z. sociatus, P. variabilis,* and *A. pulchella,* 28 to 137 days (Steen and Muscatine, 1984; Muller-Parker, 1984, 1985, 1987; Wilkerson *et al.*, 1983).

The density of algal symbionts in both *B. antilliensis* and *B. globulifera* was less than half that reported for

uration irradiances ($I_{0.95}$). The total daily net carbon fixed (P_Z^N) by the algal symbionts of *B. antilliensis* was greater than for *B. globulifera*. However, the calculated amount of fixed carbon available for translocation to the host (T) did not vary between the two species, reflecting the similar-sized algal populations and peak mitotic indices (Table I). The overall potential contribution made by translocated symbiont carbon to the daily respiratory carbon requirements of the host animal (CZAR) was slightly greater in *B. antilliensis* (109%) than in *B. globulifera* (92%).

Discussion

This study discloses significant differences in both morphological and behavioral aspects of two tropical species of the symbiotic sea anemone *Bunodeopsis;* these differences appear to be related to the possession of intracellular algal symbionts. The algal symbionts found in both species of *Bunodeopsis* were closely similar to other algae of the genus *Symbiodinium* (Blank, 1987). The symbionts from the two host species did not differ in morphology, ultrastructure, or cell-cycle timing. However, further identification of these symbionts would require the use of molecular genetic techniques (Rowan and Powers, 1991). Comparisons of the results from these and similar studies must be interpreted with caution due to possible variation in the relationships between different host species and their symbionts.

The symbionts from *B. antilliensis* and *B. globulifera* were of a similar size (7 to 10 μm in diameter) to those from other tropical sea anemones (Muller-Parker, 1984, 1985; Steen and Muscatine, 1984; Smith, 1986; Cook *et al.*, 1988). The symbionts from both species of *Bunodeopsis* had a phased cell division cycle, with peak mitotic indices occuring during the early morning, characteristic
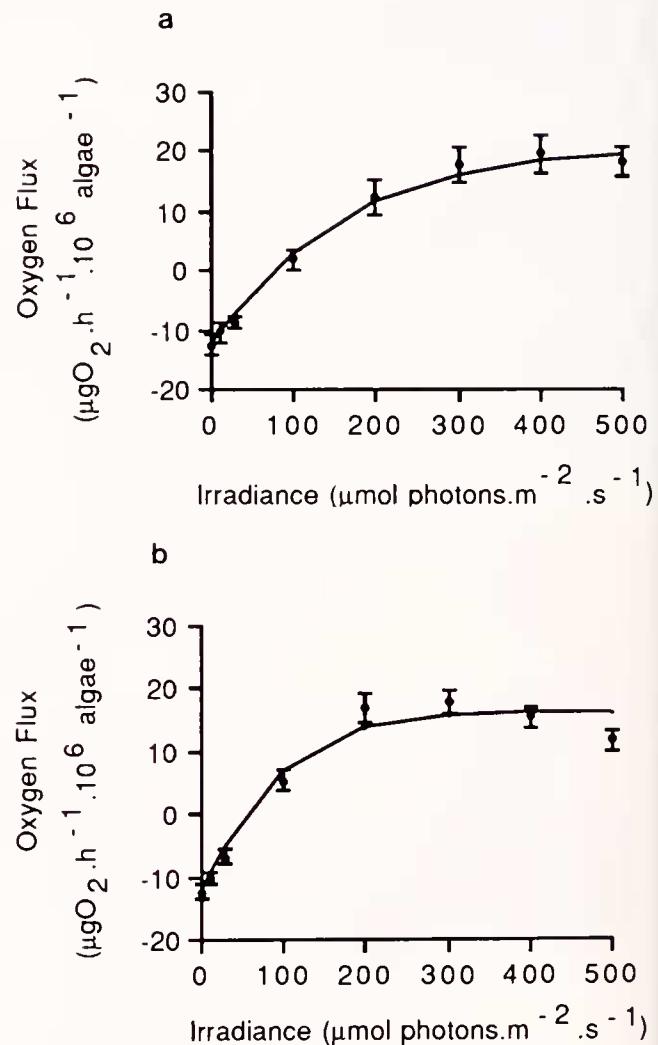
a

b

Figure 9. Net oxygen flux of the symbiotic association as a function of irradiance for (a) *Bunodeopsis antilliensis* (N = 10) and (b) *B globulifera* (N = 12). Values are means ± SE.

Table III

P-I curve parameters (means ± SE) and CZAR of Bunodeopsis antilliensis *and* B. globulifera

Parameter		Method*	B. globulifera (N = 12)	B. antilliensis (N = 10)
Anemone respiration rate ($r_{Anemone}$)	($\mu gO_2 \cdot 10^6$ algae$^{-1} \cdot$ h^{-1})	M	-12.3 ± 1.1	-12.4 ± 1.6
Anemone respiration rate ($r_{Anemone}$)	($\mu gC \cdot$ mg protein$^{-1} \cdot$ h^{-1})	D	2.25	2.43
Animal respiration rate (R_A)	($\mu gC \cdot$ mg protein$^{-1} \cdot$ 24 h^{-1})	D	52.9	57.2
Algal respiration rate (r_Z)	($\mu gC \cdot$ mg protein$^{-1} \cdot$ h^{-1})	D	0.045	0.049
Gross photosynthetic capacity (p_{Max}^G)	($\mu gO_2 \cdot 10^6$ algae$^{-1} \cdot$ h^{-1})	D	28.6 ± 2.61	34.2 ± 4.67
Gross photosynthetic capacity (p_{Max}^G)	($\mu gC \cdot$ mg protein$^{-1} \cdot$ h^{-1})	M	5.95	7.58
Net carbon fixed by algae (P_Z^N)	($\mu g \cdot$ mg protein$^{-1} \cdot$ 24 h^{-1})	D	50.2	64.0
Carbon translocation (%)		D	97.4	96.9
CZAR (%)		D	92.4	108.5

* M = measured; D = derived.

several tropical species of *Aiptasia,* 1.6 to 3.5 × 10^6 cells · mg protein^{-1} (Steele, 1976; Svoboda and Porrmann, 1980; Muller-Parker, 1984, 1987; Clayton and Lasker, 1984; Cook *et al.,* 1988). *B. antilliensis* and *B. globulifera* displayed distinct morphological adaptations that seem to be related to the possession of symbionts. The most striking morphological features of these species are the vesicles, which are considerably larger in *B. antilliensis* than in *B. globulifera.* The relative importance of these auxiliary structures (Sebens and deReimer, 1977) for exposure of the symbionts to light was evident from the marked differences in the distribution of symbionts within the two host species. In *B. globulifera* the distribution of symbionts was relatively uniform, whereas in *B. antilliensis* the vesicle-containing scapus region had the highest proportion of symbionts, with a symbiont density approximately three times higher than in the tentacles. Based on areal densities, Sebens and deReimer (1977) found the symbiont density in the scapus region of *B. antilliensis* to be 12 times higher than that in the tentacles. Similar relationships between symbiont densities and auxiliary structures were found in the tropical corallimorphs *Lebrunia coralligens* and *L. danae,* with symbiont densities three or four times higher in the pseudotentacles than in the feeding tentacles (Sebens and deReimer, 1977), and in *Discosoma sanctithomae,* with symbiont densities four times higher in the diskal tentacles than in the oral disk margin (Elliott and Cook, 1989).

Linked to the differences in anemone morphology and distribution of symbionts are significantly different patterns of light-related expansion and contraction behavior. This behavior by the host can control exposure of the symbionts to light and consequently their photosynthetic capacity (Shick and Dykens, 1984). Under illumination, vesicle inflation by *B. antilliensis* and simultaneous vesicle inflation and tentacle expansion by *B. globulifera* were indicative of mechanisms to promote exposure of their

symbiont populations to light. Vesicle inflation may also enhance the supply of carbon dioxide, because photosynthesis by symbiotic algae *in situ* may be carbon-dioxide-limited (Muscatine *et al.,* 1989). The role of algal symbionts in light-related behavioral responses is clearly seen in other tropical species that concentrate their symbionts in auxiliary structures (Gladfelter, 1975; Sebens and deReimer, 1977; Steele and Goreau, 1977; Lewis, 1984; Elliott and Cook, 1989; Shick *et al.,* 1991). Contraction by *B. globulifera* under high midday irradiances may result in shading of the symbionts in the tentacles, thereby decreasing symbiont photosynthesis and the potential for oxygen toxicity occuring in the host tissues (Shick and Dykens, 1984). In contrast, contraction of cold-stripped *B. globulifera* under low irradiances may be due to their reduced symbiont densities in comparison to untreated anemones. Continual expansion of small, or juvenile, *B. antilliensis* under illumination may reflect the relatively
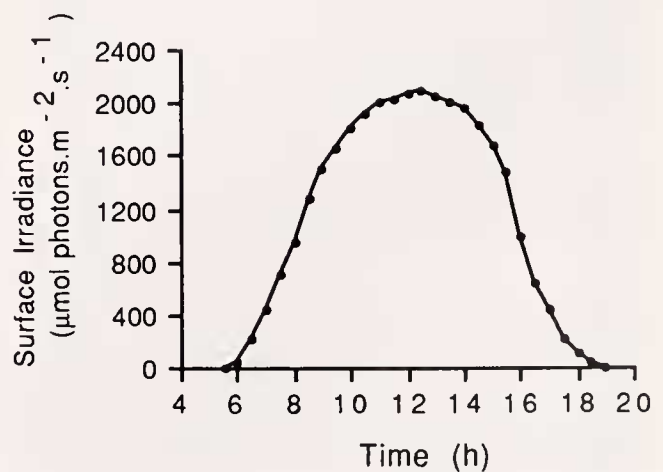

Figure 10. The diel cycle of irradiance at Discovery Bay (22 Feb.1990).

small energy saving resulting from contraction of a small compared to a large anemone (Robbins and Shick, 1980).

B. antilliensis and *B. globulifera* had expanded feeding tentacles at night, corresponding with the diel migration of zooplankton prey within Discovery Bay (Ohlhorst, 1982). Capture of more prey by *B. globulifera* than *B. antilliensis* during the day was presumably a direct consequence of their differences in light-related behavior. The present study of prey capture by *B. antilliensis* and *B. globulifera* is one of the few reported for tropical sea anemones (reviewed in Shick, 1991). These results emphasize the importance of nighttime sampling, with short intervals, because sea anemones may rapidly digest their prey (Sebens and Koehl, 1984; Zamer, 1986). Therefore, the apparent lack of prey found within the tropical corallimorph *Discosoma sanctithomae* (Elliott and Cook, 1989) and the coral *Porites porites* (Edmunds and Davies, 1989) may be artifacts due to the choice of sampling time and interval.

In association with differences in host habitat, morphology, and behavior, the algal symbionts exhibit responses to enhance photosynthesis and maximize cell division rates in low light environments (Prézelin, 1987). Variations in the photosynthetic pigment content and physiology between the symbionts from *B. antilliensis* and *B. globulifera* were indicative of responses to the different irradiance regimes of their respective host habitats. Characteristic of adaptation to lower habitat irradiances, symbionts from the deeper species *B. globulifera* had a higher chlorophyll *a* content than those from *B. antilliensis*. The chlorophyll *a* content of these symbionts was comparable to that of the symbionts from the tropical sea anemone *Aiptasia pallida*, 2 to 5 pg·cell^{-1} (Clayton and Lasker, 1984; Cook *et al.*, 1988; Lesser and Shick, 1989), but higher than for the symbionts from *A. pulchella*, 1.5 pg·cell^{-1} (Muller-Parker, 1984). The ratio of chlorophylls $a:c_2$ was lower for symbionts of *B. antilliensis* and *B. globulifera* than from *A. pulchella*, 2.2 to 3.7 (Muller-Parker, 1984, 1985, 1987). This was due to higher levels of chlorophyll c_2 in the symbionts from the two species of *Bunodeopsis* compared to those from *A. pulchella*, with 0.4 to 0.7 pg·cell^{-1} (Muller-Parker, 1984 and 1987).

The lower saturation irradiance and higher photosynthetic efficiency of symbionts from *B. globulifera* compared to those from *B. antilliensis* was also indicative of adaptation to lower light levels. These photosynthesis-irradiance responses compared well with those of symbionts from *A. pulchella* and *A. pallida* collected from habitats of differing irradiance regimes (Muller-Parker, 1984, 1985, 1987; Lesser and Shick, 1989). I_k values for symbionts from *B. antilliensis*, 238 μmol photons·m^{-2}·s^{-1}, were similar to those from *A. pulchella* collected from "sun" habitats during the summer, 288 μmol photons·m^{-2}·s^{-1} (Muller-Parker, 1987). Reflecting the differences in chlo-

rophyll *a* content, the photosynthetic efficiencies (means ± SE; $N = 10$) of symbionts from *B. antilliensis* and *B. globulifera*, 0.24 ± 0.02 and 0.17 ± 0.03 μgO$_2$·10^6 cells·h^{-1}/μmol photons·m^{-2}·s^{-1}, were considerably higher than those from *A. pulchella* based on algal cell numbers (0.04 to 0.06 μg O$_2$·10^6 cells·h^{-1}/μmol photons·m^{-2}·s^{-1}) (Muller-Parker, 1984, 1987), but were similar when normalized to algal chlorophyll *a* content. Also, photosynthetic capacities of the symbionts from *B. antilliensis* and *B. globulifera* were almost twice that of *A. pulchella* based on algal cell number, of 10 μgO$_2$·10^6 cells·h^{-1} (Muller-Parker, 1984), but did not differ when normalized to algal chlorophyll *a* content.

The dark respiration rates for anemones of both species of *Bunodeopsis* compared well with those for *A. pulchella*, of 8.2 μgO$_2$·mg protein·h^{-1} (Muller-Parker, 1984), and *A. pallida* (fed three times per week), of 10.7 μgO$_2$·mg protein·h^{-1} (Clayton and Lasker, 1984). However, symbiont respiration rates based on algal biomass estimations were low for both *B. antilliensis* and *B. globulifera*. Because algal cell densities in both species of *Bunodeopsis* were low, the estimated biomass ratio of algal to total protein was also low. Higher algal biomasses have been estimated for *Z. sociatus* (17%; Steen and Muscatine, 1984), *A. pulchella* (18%; Muller-Parker, 1984), *Aulactinia stelloides* (15%; Smith, 1986), and *Anthopleura elegantissima* (14%; Dykens *et al.*, 1992).

In *B. antilliensis* and *B. globulifera*, the proportion of photosynthetically fixed carbon available for animal respiration was high, similar to that for the zoanthids *Z. sociatus* (95%) and *P. variabilis* (89%; Steen and Muscatine, 1984). High rates of translocation reflect low rates of carbon utilization by the algal symbionts for their own growth (Muscatine *et al.*, 1983). Calculated translocation rates of *B. antilliensis* and *B. globulifera* were high due to high carbon-specific growth rates and correspondingly low algal population growth rates. Despite low carbon-specific growth rates of the symbionts from *Z. sociatus* and *P. variabilis*, 0.2·day^{-1}, compared to those for *B. antilliensis* and *B. globulifera*, algal population growth rates of *Z. sociatus* and *P. variabilis* were much lower, 0.02 and 0.01·day^{-1}, respectively.

The values of CZAR estimated for *B. antilliensis* (109%) and *B. globulifera* (92%) suggest that both species are potentially autotrophic with respect to carbon available for animal respiration on days with 8.5 h of saturating irradiance. Although CZAR values may be high, essential nutrients such as nitrogen and phosphorus need to be acquired by host heterotrophy (Muller-Parker *et al.*, 1988). Both *B. antilliensis* and *B. globulifera* were found to feed extensively on zooplankton prey, providing an alternative nutrient source to the supply of carbon fixed photosynthetically by the symbionts. The sea anemone *Anemonia sulcata* was also found to be potentially autotrophic, with

CZAR estimated to be 116%, for freshly collected Mediterranean anemones experiencing 10 h of saturating irradiance daily (Stambler and Dubinsky, 1987). However, CZAR values for the tropical zoanthids *Z. sociatus* (48%) and for *P. variabilis* (13%) (Steen and Muscatine, 1984), were considerably lower than for *B. antilliensis* and *B. globulifera*, reflecting relatively low photosynthetic capacities of the symbionts and high rates of animal respiration in the zoanthids. CZAR calculated for the temperate anemone *Anthopleura elegantissima* was also low (assuming a translocation value of 90%), 17% for high shore and 40% for low shore anemones (Shick and Dykens, 1984; Zamer and Shick, 1987). Therefore, further investigations are required to assess the influence of host habitat and locality on the nutritional contribution made by the algal symbionts to their hosts.

In summary, despite morphological differences between *B. antilliensis* and *B. globulifera*, light-related behavior correlated with the distribution of symbionts within these two host species appears to maximize exposure of the symbiont populations to light. Although *B. antilliensis* and *B. globulifera* experience different irradiance regimes due to host habitat and posture, compensatory responses by the symbionts enhance light absorption and photosynthesis. *B. antilliensis* and *B. globulifera* display two distinct strategies, combining anemone morphology with symbiont distribution and photosynthetic physiology, that result in a similar contribution made by symbiont photosynthesis to animal respiration.

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